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Interleukin-23 and T helper 17-type responses in intestinal inflammation: from cytokines to T-cell plasticity

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Summary

Interleukin-23 (IL-23) plays an essential role in driving intestinal pathology in experimental models of both T-cell-dependent and innate colitis. Furthermore, genome-wide association studies have identified several single-nucleotide polymorphisms in the IL-23 receptor (IL-23R) gene that are associated with either susceptibility or resistance to inflammatory bowel disease in humans. Although initially found to support the expansion and maintenance of $CD4^+$ T helper 17 (Th17) cells, IL-23 is now recognized as having multiple effects on the immune response, including restraining $Foxp3^+$ regulatory T-cell activity and inducing the expression of Th17-type cytokines from non-T-cell sources. Here we focus on Th17 cells and their associated cytokines IL-17A, IL-17F, IL-21 and IL-22. We review studies performed in mouse models of colitis where these effector cytokines have been shown to have either a pathogenic or a tissue-protective function. We also discuss the heterogeneity found within the Th17 population and the phenomenon of plasticity of Th17 cells, in particular the ability of these lymphocytes to extinguish IL-17 expression and turn on interferon-γ production to become Th1-like 'ex-Th17' cells. Interleukin-23 has been identified as a key driver in this process, and this may be an additional mechanism by which IL-23 promotes pathology in the intestinal tract. These 'ex-Th17' cells may contribute to disease pathogenesis through their secretion of pro-inflammatory mediators.

Keywords: colitis; inflammation; interferon-γ; interleukin-17A; interleukin-23; T helper 17 cells

Interleukin-23 plays a crucial role in driving intestinal inflammation

Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, is a chronic inflammatory condition of the gastrointestinal tract caused in part by an inappropriate immune response to the intestinal microflora.[1] Cells of both the innate and adaptive arms of the immune response are believed to participate in the inflammatory reaction by secreting pro-inflammatory cytokines, each with multiple downstream targets and effects. Early studies in experimental models using genetic ablation or monoclonal antibody (mAb) neutralization of the interleukin-12 (IL-12) p40 subunit *in vivo* suggested that IL-12 and a subsequent T helper 1 (Th1)-type response played a crucial role in colitis pathogenesis.[2] However, with the discovery in 2000 by Oppmann *et al.*[3] of a new p19 subunit that can pair with p40 to form the cytokine IL-23, previous studies using anti-p40 mAb had to be re-evaluated to examine if indeed IL-12 or IL-23 was driving the colitic response. Consequently, in several models of colitis, it is now clear that IL-23 is the major driver of intestinal inflammation.[4–8] Importantly, genome-wide association studies of large cohorts of patients with IBD and healthy controls subsequently identified several single-nucleotide polymorphisms in the IL-23 receptor (IL-23R) gene locus associated with either susceptibility or resistance to IBD,[9,10] strongly arguing that IL-23 is of importance for disease pathogenesis also in human IBD. The molecular mechanism(s) by which certain IL-23R single-nucleotide polymorphisms correlate with IBD susceptibility and others with resistance is not completely understood, but possible explanations include enhanced versus reduced signalling through the receptor

(gain or loss of function) caused by altered ligand binding. In addition, certain IL-23R mutations have been shown to cause alternative mRNA splicing, leading to the formation of truncated forms of the receptor, including a soluble form that can act as a decoy receptor,[11,12] thereby preventing IL-23 from exerting its effects on target cells.

Interleukin-23 has broad effects on both T cells and non-T cells

Interleukin-23 is produced by activated dendritic cells and macrophages in response to microbial stimulation (reviewed by Langrish *et al.*[13]), but the exact mechanism by which this cytokine contributes to the inflammatory response in the intestine is not yet clear. Much attention has focused on the role of IL-23 in expanding/maintaining $CD4^+$ T cells of the Th17 subset[14–16] (Fig. 1), a population characterized by its production of IL-17A, IL-17F, IL-21 and IL-22[16,17] and reported to play a pathogenic role in animal models of autoimmune disease such as collagen-induced arthritis and experimental autoimmune encephalomyelitis (EAE).[18] $CD4^+$ Th17 cells have also been implicated in multiple autoimmune and inflammatory disorders in humans,[19] and elevated levels of IL-17A, IL-17F, IL-21 and IL-22 have been found in the inflamed gut in both human IBD and experimental models of the disease.[6,7,20–25] The murine intestine harbours a large proportion of $CD4^+$ Th17 cells in steady-state,[26] possibly reflecting the constitutive IL-23 expression found in the terminal ileum of healthy mice.[27] Moreover, specific constituents of the gut microbiota, e.g. segmented filamentous bacteria, have been reported to induce this IL-17-secreting $CD4^+$ T-cell subset.[28–30]

Interleukin-23 has also been reported to inhibit the accumulation of intestinal $Foxp3^+$ regulatory T (Treg) cells, a population of $CD4^+$ T cells of importance for maintaining intestinal homeostasis.[31] Hence, using the T-cell transfer model of colitis, in which disease is induced in T-cell-deficient $Rag^{-/-}$ mice by transfer of naive wild-type $CD45RB^{high}$ $CD4^+$ T cells, Izcue *et al.*[32] have demonstrated increased frequencies of intestinal $Foxp3^+$ cells if the $Rag^{-/-}$ recipients are unable to produce IL-23. Similarly, in the absence of IL-23R on donor T cells, $Rag^{-/-}$ recipients show increased frequencies of colonic $Foxp3^+$ cells, indicating that IL-$23R^{-/-}$ T cells are better than wild-type T cells at developing into inducible $Foxp3^+$ Treg (iTreg) cells, and that IL-23 inhibits iTreg cell differentiation (Fig. 1).[33] In the latter study, the authors also provided evidence for a cell-extrinsic effect of IL-23 in reducing T-cell IL-10 production. Hence, whereas high IL-10 expression was observed from T cells isolated from the colon of $Rag^{-/-}$ mice given IL-$23R^{-/-}$ $CD45RB^{high}$ $CD4^+$ cells alone, this enhanced IL-10 expression was abrogated by co-transfer with wild-type T cells, suggesting that IL-23-responsive wild-type cells can reduce the ability of IL-$23R^{-/-}$ T cells to produce IL-10[33]. These findings indicate that IL-23 may promote intestinal inflammation by constraining tissue-protective populations and functions of T cells (Fig. 1).

As IL-23 blockade has beneficial effects also in innate colitis,[5,6] this cytokine is believed to, in addition, act on non-T cells (Fig. 1). Indeed, a recent report by Buonocore *et al.*[34] has demonstrated the presence of a Lin^- $Thy1^{high}$ $SCA\text{-}1^+$ $ROR\gamma t^+$ innate lymphoid population that secretes IL-17A, IL-22, and interferon-γ (IFN-γ) in response to IL-23. The authors showed that these innate lymphoid cells are found at low frequencies in the normal mouse gut, but that they increase significantly during intestinal inflammation. Furthermore, mAb depletion of $Thy1^+$ cells in 129SvEv $Rag^{-/-}$ mice receiving *Helicobacter hepaticus* or in C57BL/6 $Rag^{-/-}$ animals given anti-CD40 mAb prevented the development of colitis, suggesting that the

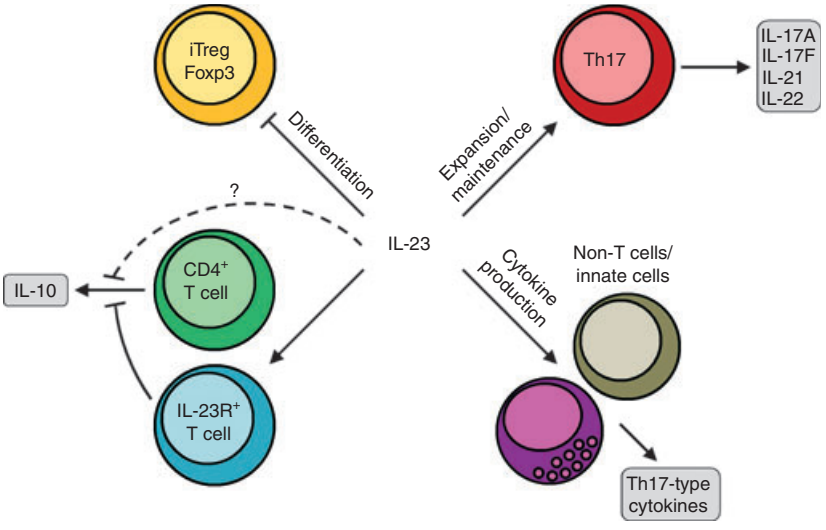

Figure 1. Cartoon summarizing functions of interleukin-23 (IL-23) that may contribute to colitis pathogenesis. IL-23 has been shown to promote the expansion/maintenance of $CD4^+$ T helper 17 (Th17) cells (top right), induce the production of Th17-type cytokines by non-T cells/innate cells (bottom right), and inhibit the generation of inducible $Foxp3^+$ regulatory T (iTreg) cells (top left). In addition, IL-23 has been reported to reduce IL-10 production in a cell-extrinsic manner by acting on IL-$23R^+$ T cells to inhibit IL-10 secretion by other $CD4^+$ T cells (bottom left). Whether IL-23 can act directly on T cells to inhibit IL-10 production is currently unknown (broken arrow).

IL-23-responsive innate lymphoid cells contribute to the inflammatory cascade in these T-cell-independent models of colitis.[34] Importantly, the authors reported a similar population of IL-23-responsive IL-17-secreting innate lymphoid cells in the inflamed intestine of patients with IBD.[34] Taken together, IL-23 can contribute to intestinal inflammation in multiple ways, from restraining Foxp3$^+$ Treg-cell activity to inducing the expression of Th17-type cytokines from both T cells and non-T-cell sources (Fig. 1). Table 1 summarizes known cellular sources of Th17-type cytokines.

Host-protective versus pathogenic roles of Th17-type cytokines in the gut

With elevated levels of Th17-type cytokines in the colitic gut, a lot of effort has gone into elucidating their individual role(s) in the intestine in health and disease. What has become clear is that Th17-associated cytokines play both host protective and pathogenic functions at mucosal sites. The host protective roles can be divided into (i) elimination of pathogens[35,36] and (ii) tissue-protective functions. When it comes to host defence against microbes in the intestinal tract, IL-17A, IL-17F and IL-22 have all been shown to be important for the control of oral *Citrobacter rodentium* infection, as mice deficient in these cytokines show enhanced *C. rodentium* burdens in the colon (IL-17A$^{-/-}$, IL-17F$^{-/-}$ and IL-17A$^{-/-}$ IL-17F$^{-/-}$ mice)[37] or mesenteric lymph nodes, spleen and liver (IL-22$^{-/-}$ mice)[38] compared with wild-type animals. The elevated bacterial burdens were associated with reduced levels of colonic β-defensins 1, 3 and 4 in IL-17A$^{-/-}$, IL-17F$^{-/-}$ and IL-17A$^{-/-}$ IL-17F$^{-/-}$ mice[37] and RegIIIβ and RegIIIγ in IL-22$^{-/-}$ animals,[38] in agreement with the reported role of these cytokines in inducing the expression of antimicrobial peptides.[39,40]

Perhaps the best example of the tissue-protective effects of Th17-type cytokines in the gut is that of neutralization of IL-17A, either by mAb treatment or by genetic ablation, which leads to exacerbated intestinal inflammation in the dextran sulphate sodium (DSS) colitis model.[41,42] When administered to mice for a few days via the drinking water, DSS triggers an acute inflammatory response by 'mechanical' disruption and injury to the epithelial layer, leading to a rapid transient weight loss, normally followed by recovery. (For a review of different experimental models of intestinal inflammation, see Strober *et al.*[43]) Interleukin-17A is believed to exert its host-protective effect in this model through strengthening tight-junction formation by inducing the expression of claudins in intestinal epithelial cells[44] and by stimulating mucin production,[45] thereby increasing mucosal barrier function (Table 2). Similarly, compared with wild-type mice, animals deficient in IL-22 show increased weight loss and higher mortality rates following DSS administration, suggesting a tissue-protective role for this cytokine.[46] Indeed, IL-22 has been shown to stimulate intestinal epithelial cell proliferation[24] and enhance goblet cell restitution and mucus production,[47] both functions that would promote intestinal barrier integrity (Table 2).

In contrast to the tissue-protective effects of IL-17A and IL-22, mice deficient in IL-17F develop milder disease symptoms than wild-type animals when given DSS,[42] suggesting that IL-17F exacerbates inflammation in this model. Indeed, DSS-fed IL-17F$^{-/-}$ animals display reduced chemokine mRNA expression in the colon compared with similarly treated wild-type controls.[42] The reason why IL-17A$^{-/-}$ and IL-17F$^{-/-}$ mice show different disease outcomes following DSS administration remains unclear. Both cytokines bind receptors on myeloid and endothelial cells, and induce the expression of IL-1β, IL-6, tumour necrosis factor-α (TNF-α) and chemokines involved in neutrophil recruitment.[48,49] Furthermore, IL-17A and IL-17F both signal through the same receptor subunits IL-17RA and IL-17RC (reviewed by Gaffen[50]). The precise structure of the receptors for IL-17A and IL-17F still remains to be determined, and it is possible

Table 1. Cellular sources of T helper 17 (Th17)-type cytokines

Cytokine	Cellular source	References
IL-17A	CD4$^+$ T cells	14,15,69–71,103–108
	CD8$^+$ T cells	107,109,110
	$\gamma\delta$ T cells	107,111–118
	Tfh cells	119
	NKT cells	120–124
	Monocytes/macrophages	6,20,125
	Granulocytes/neutrophils	6,126,127
	LTi$^-$ like cells	34,128–131
	Lin$^-$ innate lymphoid cells	34
	Paneth cells	132
	Mast cells	133
IL-17F	CD4$^+$ T cells	40,62,64,134,135
	$\gamma\delta$ T cells	117,118
	Epithelial cells	37,136
IL-17F/A	CD4$^+$ T cells	51,52,137
IL-21	CD4$^+$ T cells	62–64,72,138,139
	Tfh cells	119,140,141
	NKT cells	142,143
IL-22	CD4$^+$ T cells	40,74,144–147
	CD8$^+$ T cells	74
	$\gamma\delta$ T cells	74,115
	NK-like cells	46,148–150
	Monocytes	74
	CD11c$^+$ DCs[a]	38
	LTi-like cells	129–131
	Lin$^-$ innate lymphoid cells	34

DCs, dendritic cells; LTi, lymphoid tissue inducer cell; NK, natural killer cell; NKT, natural killer T cell; Tfh, T follicular helper cell.
[a]CD11c is also expressed on NK and LTi cell populations (reviewed in 151).

Table 2. T helper 17-associated cytokines in colitis

Cytokine	Non-pathogenic or tissue protective	Pathogenic	Possible mechanism(s) of tissue protective versus pathogenic effects
IL-17A	Anti-IL-17A mAb treatment exacerbates DSS colitis[41] IL-17A$^{-/-}$ mice show exacerbated DSS colitis[42] IL-17A$^{-/-}$ CD45RB^{high} CD4^{+} T cells induce severe colitis indistinguishable from that induced by wild-type T cells[32,65,66] or an accelerated wasting disease with some markers of more severe colitis compared to that induced by wild-type T cells[67] Anti-IL-17A mAb treatment fails to block colitis induced by wild-type CD45RB^{high} CD4^{+} T cells[66]		Improved barrier function by: Induction of claudin expression leading to strengthened tight junctions[44] Induction of mucin production[45] Pro-inflammatory by induction of neutrophil-attracting chemokines, IL-1β, IL-6, TNF-α, G-CSF, and GM-CSF, and MMPs[49]
IL-17F	IL-17F$^{-/-}$ CD45RB^{high} CD4^{+} T cells induce severe colitis indistinguishable from that induced by wild-type T cells[66]	IL-17F$^{-/-}$ mice develop less severe DSS colitis[42]	Pro-inflammatory by induction of neutrophil-attracting chemokines, IL-1β, IL-6, TNF-α, G-CSF, and GM-CSF, and MMPs[49]
IL-21		IL-21$^{-/-}$ mice develop less severe DSS and TNBS colitis, and IL-21R-Fc ameliorates DSS colitis[55]	Pro-inflammatory: Induction of MMP production by intestinal fibroblasts[56] Induction of MIP-3α/CCL20 expression by colonic epithelial cells[57] Renders CD25^{-} CD4^{+} T cells resistant to Treg-mediated suppression[58,59] Enhances IFN-γ production from T cells and NK cells[60,61] Induces Th17 cells[62–64]
IL-22	IL-22$^{-/-}$ mice show increased weight loss and mortality in DSS colitis[46] IL-22$^{-/-}$ CD45RB^{high} CD4^{+} T cells induce severe colitis indistinguishable from that induced by wild-type T cells[66] IL-22$^{-/-}$ CD45RB^{high} CD4^{+} T cells induce more severe colitis in IL-22$^{-/-}$ Rag$^{-/-}$ than in IL-22$^{+/+}$ Rag$^{-/-}$ recipients[46]		Improved barrier function by: Induction of epithelial cell proliferation[24] Enhances goblet cell restoration and mucus production[47]

DSS, dextran sodium sulphate; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; IL-21R-Fc, interleukin-21 receptor Fc fusion protein; MMP, matrix metalloproteinase; TNBS, trinitrobenzene sulphonic acid; TNF-α, tumour necrosis factor-α.

that ligand affinity, downstream signalling cascades, and receptor tissue distribution could explain the difference in intestinal pathology observed in IL-17A$^{-/-}$ and IL-17F$^{-/-}$ mice following DSS administration.[49,50] Besides IL-17A and IL-17F (which exist as homodimers), there are also reports of IL-17F/A heterodimers, adding to the complexity by which these cytokines exert their effects on target cells.[51,52] Of note, mice deficient in IL-17RA or its downstream adaptor protein Act1 show reduced intestinal inflammation in response to DSS or trinitrobenzene sulphonic acid (TNBS),[53,54] a hapten reagent that triggers an inflammatory reaction following intrarectal administration in 50% ethanol to break the epithelial barrier. These findings indicate that the IL-17F-driven inflammatory response may dominate over the tissue-protective actions of IL-17A in these two models of acute colitis.

Another Th17-type cytokine that plays a pathogenic role in DSS and TNBS colitis is IL-21. Hence, IL-21$^{-/-}$ mice challenged with either of these compounds develop less inflammation compared with wild-type controls, and administration of an IL-21R-Fc fusion protein attenuates colitis in wild-type animals given DSS.[55] Interleukin-21

has a wide variety of effects that may contribute to its inflammatory role in the intestine. For example, IL-21 induces the production of tissue-degrading matrix metalloproteinases from intestinal fibroblasts,[56] stimulates the synthesis of the T-cell chemoattractant macrophage inflammatory protein-3α (MIP-3α)/CCL20 from colonic epithelial cells,[57] renders $CD25^{-}$ $CD4^{+}$ T cells resistant to Treg-mediated suppression,[58,59] and in some cases enhances IFN-γ production from T cells and natural killer (NK) cells[60,61] (Table 2). Moreover, T-cell-derived IL-21 is thought to act in an autocrine fashion to induce Th17 cells.[62–64] Taken together, studies in acute chemical-induced intestinal inflammation, such as that triggered by DSS or TNBS where epithelial barrier function is disrupted, have illustrated that different Th17-type cytokines play distinct roles, some being tissue protective (IL-17A and IL-22) and others pathogenic (IL-17F and IL-21).

To begin to define the role of T-cell versus non-T-cell-derived Th17-type cytokines in colitis pathogenesis, several groups have used the $CD45RB^{high}$ transfer model where donor $CD4^{+}$ T cells and/or $Rag^{-/-}$ recipients can be made deficient for individual cytokines. To date, the majority of studies have used cytokine-deficient $CD45RB^{high}$ $CD4^{+}$ T cells and transferred these into normal cytokine-sufficient $Rag^{-/-}$ hosts. As such, IL-$17A^{-/-}$ $CD45RB^{high}$ $CD4^{+}$ cells have in three independent reports been shown to cause severe colitis indistinguishable from that induced by wild-type $CD45RB^{high}$ $CD4^{+}$ cells,[32,65,66] demonstrating that T-cell-derived IL-17A is dispensable for colitis induction. Similarly, a fourth study showed minor differences in intestinal pathology between $Rag^{-/-}$ recipients of wild-type versus IL-$17A^{-/-}$ $CD45RB^{high}$ $CD4^{+}$ cells, but reported an accelerated wasting disease in recipients of IL-17A-deficient T cells,[67] possibly reflecting a lack of T-cell IL-17A-mediated protective effects systemically.

Similar to findings using IL-$17A^{-/-}$ $CD45RB^{high}$ cells, T-cell-derived IL-17F or IL-22 is not required for colitis pathogenesis in the T-cell transfer model, as $CD45RB^{high}$ $CD4^{+}$ cells isolated from IL-$17F^{-/-}$ or IL-$22^{-/-}$ animals induce colitis indistinguishable from that caused by wild-type cells.[66] In a separate study, IL-22 was even shown to play a protective role in the transfer model of colitis. Hence, using various combinations of $CD45RB^{high}$ T cells and $Rag^{-/-}$ hosts with or without the capacity to produce IL-22, the most severe intestinal pathology was observed when both donor $CD45RB^{high}$ cells and recipient $Rag^{-/-}$ mice lacked IL-22.[46] These observations suggest that both T-cell and non-T-cell sources of IL-22 are tissue protective in T-cell transfer colitis. The authors went on to show that NK cells are the likely innate source of the disease-protective IL-22 in this model.[46]

In contrast to the acute colitis models of DSS and TNBS where a protective or pathogenic role of individual Th17-type cytokines has been easier to reveal, chronic models of colitis (such as the T-cell transfer model) appear more complex and require blockade of multiple cytokines to affect disease pathogenesis. In line with the studies mentioned above in which T-cell-derived IL-17A was shown to be dispensable for T-cell-transfer colitis,[32,65–67] depletion of this cytokine by anti-IL-17A mAb treatment of $Rag^{-/-}$ mice given wild-type $CD45RB^{high}$ $CD4^{+}$ cells had no effect on the inflammatory response.[66] In contrast, in the same study, colitis was ameliorated when anti-IL-17A mAb was given to $Rag^{-/-}$ recipients of IL-$17F^{-/-}$ $CD45RB^{high}$ $CD4^{+}$ cells,[66] suggesting redundant pathogenic effects of IL-17A and IL-17F in this model. Similarly, $Rag^{-/-}$ recipients of IL-$17A^{-/-}$ $CD45RB^{high}$ $CD4^{+}$ cells show a reduction in intestinal inflammation when given anti-IL-6R mAb,[65] and combined treatment with anti-IL-17A plus anti-IL-6 mAb significantly ameliorates the severity of intestinal inflammation in $Rag^{-/-}$ mice given wild-type $CD45RB^{high}$ $CD4^{+}$ cells plus IL-23.[4] Together, these findings suggest that blocking individual Th17-associated cytokines is not enough to see a beneficial effect in chronic colitis; instead, it is necessary to target multiple pathways or mediators involved in either the Th17 response or in Th17 cell development. Consequently, learning more about the function and differentiation of Th17 lymphocytes is vital to the development of therapeutic treatments of intestinal inflammation.

Th17 cells, a heterogeneous and plastic population

While cells of the Th17 lineage are able to produce IL-17A, IL-17F, IL-21 and IL-22,[16,17] it has become clear that at the single-cell level, not all cells secrete all these cytokines simultaneously. Instead, Th17 cells are a heterogeneous population containing a number of different subpopulations expressing different combinations of Th17-type cytokines.[40,68] The cytokine profile of an individual Th17 cell is likely to be influenced by the local environment during T-cell priming. Hence, activation of murine $CD4^{+}$ cells in the presence of transforming growth factor-β (TGF-β) + IL-6[26,69–71] or TGF-β + IL-21,[63,64] or of human $CD4^{+}$ cells in the presence of IL-1β + IL-6[72] or IL-1β or IL-23 alone,[73] triggers T-cell IL-17A and IL-17F secretion. TGF-β does not seem to be required for IL-22 production though; instead this cytokine is induced by IL-6 or IL-23.[74] Th17 cells have also been shown to produce TNF-α and IL-6,[15] and one study has reported the expression of IL-10 by Th17 cells generated with TGF-β and IL-6, a phenotype not observed when cells were expanded with IL-23.[75] In addition, much attention has recently focused on cells co-expressing IL-17A and IFN-γ, a cytokine normally associated with Th1 cells, adding to the complexity of Th17 cell heterogeneity. These IFN-γ/IL-17 double-producing cells are found in

both mice and humans, often associated with infection or isolated from sites of inflammation.[68,76–80] In the case of the intestine, IFN-γ^+ IL-17A$^+$ cells have been isolated from the inflamed gut of patients with Crohn's disease.[78,79] Much attention is currently focusing on elucidating the role of IFN-γ^+ IL-17A$^+$ cells in various inflammatory diseases. A recent report by Ahern *et al.*[33] has demonstrated that CD45RB^{high} CD4$^+$ T cells lacking the IL-23R fail to develop into IFN-γ/IL-17A double producers and do not trigger colitis in the T-cell transfer model, suggesting that these cells develop through an IL-23-dependent pathway and may play an important role in disease pathogenesis.

Apart from their apparent IL-23 dependence, so far little is known about how IFN-γ^+ IL-17A$^+$ CD4$^+$ T cells arise. However, over the last few years it has become apparent that cells of the Th17 lineage are plastic and fail to maintain a stable phenotype when cultured *in vitro* or when transferred *in vivo* to recipient mice,[81,82] in certain situations acquiring the ability to secrete additional cytokines (e.g. IFN-γ). This Th17 phenotype instability was discovered initially using TCR transgenic CD4$^+$ T cells polarized *in vitro* towards Th17 cells,[83–86] and more recently with highly purified IL-17A$^+$ or IL-17F$^+$ populations isolated by cytokine-capture assays[85,87,88] or by cell sorting based on surface expression of reporters (such as Thy-1.1, red fluorescent protein, or enhanced yellow fluorescent protein [eYFP]) that mark cells that have activated the IL-17F[84,89,90] or IL-17A[91] programme. There are some general conclusions regarding Th17 stability that can be drawn from these studies. First, TGF-β is needed to maintain IL-17A production by *in vitro*-generated Th17 cells.[84] Second, *in vitro*-generated Th17 cells can be converted into Th1- or Th2-like cells when cultured under Th1- or Th2-polarizing conditions.[87] Moreover, both IL-12 and IL-23 can induce IFN-γ production by *in vitro*-generated Th17 cells (Fig. 2); IL-12 causes a rapid and near total suppression of the Th17 programme with simultaneous up-regulation of the Th1 transcription factor T-bet and IFN-γ, whereas IL-23 requires several rounds of stimulation to cause a moderate deviation from a Th17 to a Th1 phenotype.[84] At the mRNA level, Th17 cells deviated by IL-12 appear similar to Th1 cells, with low levels of *Rorc* and *Rora* and increased expression of *Tbx21*, *Fasl* and *Gzma*.[84] Interleukin-12 has been reported to down-regulate IL-17 expression and induce switching to IFN-γ production also in human Th17 populations, both in Th17 clones[78] and in *in vitro*-derived Th17 and IFN-γ^+ IL-17A$^+$ cells.[68] Third, the transition from Th17 cells to IFN-γ-producing cells requires signal transducer and activator of transcription 4 (STAT4) and T-bet,[84] factors that are up-regulated following culture of Th17 populations with IL-12.[84,85,88,92]

Experiments have also been performed to analyse the phenotype stability of *in-vitro*-generated Th17 cells following *in vivo* transfer. Hence, various Th17 populations have been given to Rag$^{-/-}$ or wild-type mice followed by examination of the cells at different time-points after transfer. These experiments have revealed that *in-vitro*-generated Th17 cells lose their IL-17 expression and start to secrete IFN-γ following transfer to T-cell-deficient mice.[83–85,89] One of these studies employed the T-cell transfer model and Th17 cells (purified based on Thy1.1 expression marking activation of the IL-17F programme) and showed that some of these cells when recovered from mesenteric lymph nodes of colitic Rag$^{-/-}$ recipients had extinguished IL-17 expression and switched on IFN-γ production.[84] In contrast to the Th17 phenotype switch observed in T-cell-deficient recipients, Nurieva *et al.*[89] have reported that Th17 cells maintain their phenotype when given to T-cell-sufficient wild-type mice. Together, these findings suggest that the Th17 cell phenotype is unstable in a lymphopenic environment, which promotes homeostatic proliferation, but less so in normal hosts.

In contrast to the plasticity noted for *in vitro*-derived Th17 cells, Lexberg *et al.*[87] initially reported that *in-vivo*-generated Th17 cells have a stable memory for IL-17A, because these cells keep their production of IL-17A even in the presence of IL-12 + anti-IL-4 mAb. One explanation for these findings could be that Th17 cells generated *in vitro* and *in vivo* show differences when it comes to their expression of a functional IL-12R. Hence, Bending *et al.*[85] have shown that *in-vitro*-generated Th17 lymphocytes express IL-12Rβ2 and are responsive to IL-12, whereas *in-vivo*-derived Th17 cells lack this IL-12R subunit.[88,92] Subsequent studies have shown that *ex vivo* Th17 cells can be converted to Th1-like cells *in vitro* following IFN-γ-induced up-regulation of T-bet and acquisition of IL-12Rβ2[88,92] (Fig. 2).

With the development of various reporter mice in which cells that have activated the Th17 programme are permanently marked,[84,89–91] it is now possible to examine Th17 cell phenotype stability *in vivo* in the whole animal without the need for adoptive transfer experiments. The first report where such a mouse strain has been used to examine Th17 cell fate during an inflammatory disease and after a challenge with an infectious agent is that by Hirota *et al.*[91] who made use of an IL-17A-eYFP reporter mouse (which permanently marks IL-17A$^+$ cells with eYFP) to map the fate of IL-17A-secreting cells during EAE and *Candida albicans* infection. The authors clearly demonstrate that IFN-γ-producing CD4$^+$ T cells in the draining lymph nodes and spinal cord of mice with EAE were once IL-17A producers, as identified by their expression of eYFP.[91] Moreover, the production of other pro-inflammatory cytokines such as granulocyte–macrophage colony-stimulating factor (GM-CSF), IL-2, and TNF-α by effector CD4$^+$ T cells in the spinal cord was derived almost exclusively from 'ex-Th17' cells, with no apparent contribution from Th1 cells.[91] Importantly, when the IL-

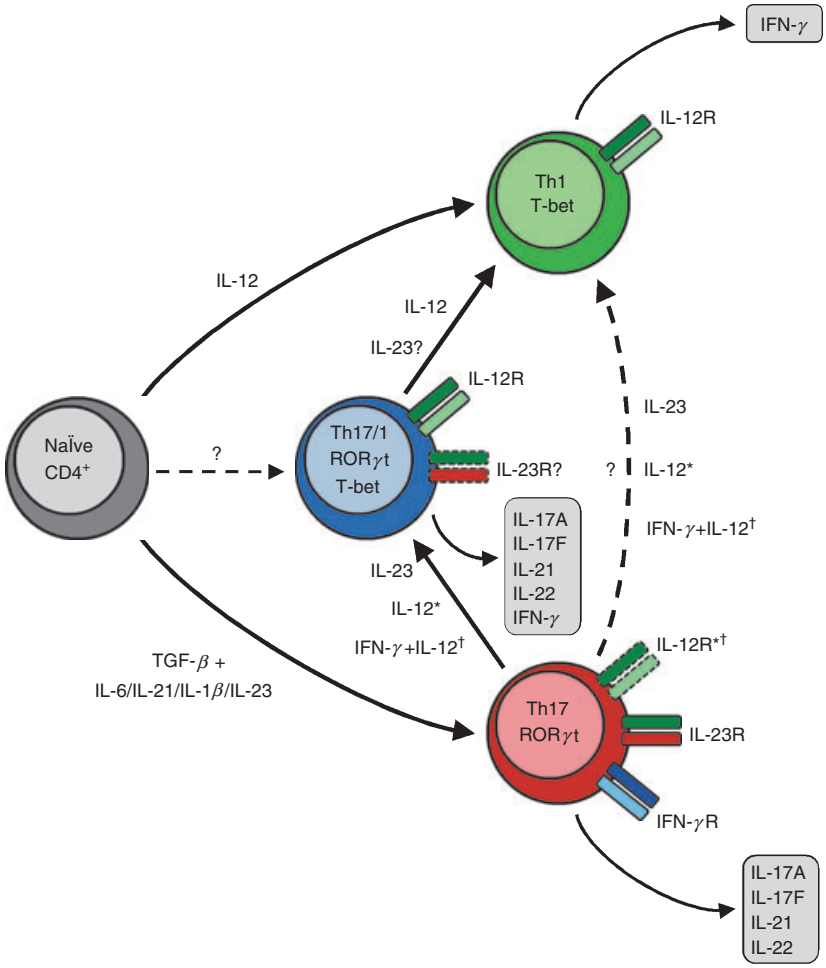

Figure 2. Interleukin-12 (IL-12) and IL-23 promote switching from a T helper 17 (Th17) to a Th1 phenotype. In the presence of polarizing cytokines, naive CD4$^+$ T cells differentiate into Th1 or Th17 subsets, each characterized by its transcription factor T-bet and RORγt, respectively, and the production of Th1- versus Th17-associated cytokines. Whereas Th1 cells represent a stable phenotype, Th17 cells are plastic and have been shown to acquire a Th1-like phenotype, up-regulating T-bet and interferon-γ (IFN-γ), in response to IL-12 or IL-23. This cartoon summarizes data from both *in vitro* and *in vivo* experiments from several laboratories (as referenced in the main text) and depicts the cytokines, cytokine receptors, transcription factors, and cell phenotypes involved in the switching process. Based on the observations of IFN-γ/IL-17A double-producing CD4$^+$ T cells, Th17 cells may switch to a Th1 phenotype via a Th17/1 intermediate stage where the cell expresses both RORγt and T-bet and produces cytokines characteristic of both Th17 and Th1 cells; however, direct switching from a Th17 to a Th1 phenotype has not yet been ruled out. The asterisk indicates that *in-vitro*-generated Th17 cells express IL-12 receptor β2 (IL-12Rβ2) and can be deviated towards a Th1 phenotype by IL-12. In contrast, as represented by the dagger (and dashed outlines on the receptor), *ex vivo* Th17 cells do not express IL-12Rβ2, but can up-regulate this receptor subunit after exposure to IFN-γ, allowing the cells to respond to IL-12 and become Th1 like. Similarly, IL-23 has been shown to cause Th17 cells to activate the Th1 programme both *in vitro* and *in vivo*. Whether Th17/1 cells can be differentiated directly from naive CD4$^+$ precursors is yet to be determined. Black text and arrows denote polarizing cytokines, and text within boxes highlighted in grey represent cytokines secreted by the different Th subsets. TGF-β, transforming growth factor-β.

17A-eYFP reporter strain was crossed onto the IL-23p19$^{-/-}$ background and mice were immunized with myelin oligodendrocyte glycoprotein peptide in complete Freund's adjuvant (MOG-CFA) to induce EAE, eYFP$^+$ T cells did not become IFN-γ$^+$, demonstrating that the switch from an IL-17A-producing T cell to an IFN-γ-secreting cell depends on IL-23 *in vivo*, subsequently shown to be the result of IL-23-driven up-regulation of T-bet.[91] In contrast to the results in the EAE model, infection of IL-17A-eYFP reporter mice with *C. albicans*, which is rapidly cleared from the host, did not give rise to eYFP$^+$ IFN-γ$^+$ cells.[91] Together, these findings indicate that Th17 cell fate is shaped by the *in vivo* microenvironment, with chronic inflammatory states promoting phenotype switching and the expression of IFN-γ and other pro-inflammatory cytokines in Th17 cells.

Are 'true' Th17 cells really pathogenic or is a switch in phenotype required for colitis pathogenesis?

Given the essential role of IL-23 in colitis pathogenesis, is there any evidence that this cytokine is driving intestinal inflammation by promoting the deviation of Th17 cells towards a Th1 phenotype, causing them to extinguish IL-17 expression and up-regulate IFN-γ and T-bet? A pro-inflammatory role for IFN-γ in the intestine has been long acknowledged, as treatment with anti-IFN-γ mAb ameliorates the development of disease in both T-cell transfer colitis[93,94] and in the spontaneous and *H. hepaticus*-triggered inflammation observed in IL-10$^{-/-}$ animals.[95,96] A link between IL-23 and IFN-γ was noted already in the original description of IL-23 by Oppmann *et al.*[3] when this cytokine was shown to stimulate IFN-γ production by human memory T cells. In addition, evidence from both experimental *Mycobacterium tuberculosis* infection and the *H. hepaticus* colitis model suggests the involvement of IL-23 in inducing CD4$^+$ T-cell IFN-γ production.[7,97,98] Hence, it is possible that the pathogenic role of IL-23 in driving colitis can be partly explained by this cytokine's ability to turn on IFN-γ production[84,91] and other pro-inflammatory mediators[91] in Th17 cells, subsequently leading to or exacerbating inflammation. Evidence from the diabetes model supports the hypothesis that a switch in cell phenotype can be of importance for pathogenicity, as islet-reactive Th17 populations convert into Th1-like cells and cause diabetes in an IFN-γ-dependent manner when transferred to non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice.[85,86] In the case of intestinal inflammation, additional properties besides IFN-γ production may determine disease-inducing potential of switched Th17 cells, as IFN-$\gamma^{-/-}$ CD4$^+$ T cells are themselves colitogenic upon transfer to T-cell-deficient hosts, although in most cases less so than wild-type CD4$^+$ cells.[7,99,100] Nevertheless, the inability of IL-23R-deficient CD4$^+$ T cells to develop into IFN-γ^+ IL-17A$^+$ cells and to induce colitis in Rag$^{-/-}$ recipients suggests that a phenotype switch may be important for disease pathogenesis also in the intestine.[33] In this case, IFN-γ may be a marker of such a switch rather than being the pathogenic factor itself. Previous studies have demonstrated that CD4$^+$ T cells deficient in either STAT4,[99] T-bet,[101] RORγt,[66] or STAT3[102] are unable to induce colitis after transfer to T-cell-deficient mice. These findings argue that these key regulators of Th1 and/or Th17 cell development are needed to induce disease; however, exactly where at the single-cell level these STAT proteins and transcription factors are required still remains to be determined. As removal of only one of the four key regulators (STAT4 or T-bet for Th1 cells, or STAT3 or RORγt for Th17 cells) renders T lymphocytes unable to trigger colitis, it is possible that a mixture of both Th1 and Th17 cells are needed for the inflammatory response, and a defect in one subset cannot be compensated for by the presence of the other subset (Fig. 3, red arrows). An alternative possibility is that these four key regulators need to be expressed in the same cell (Fig. 3, blue arrows). In this regard, it would be interesting to examine whether Th17 cells that have extinguished their IL-17

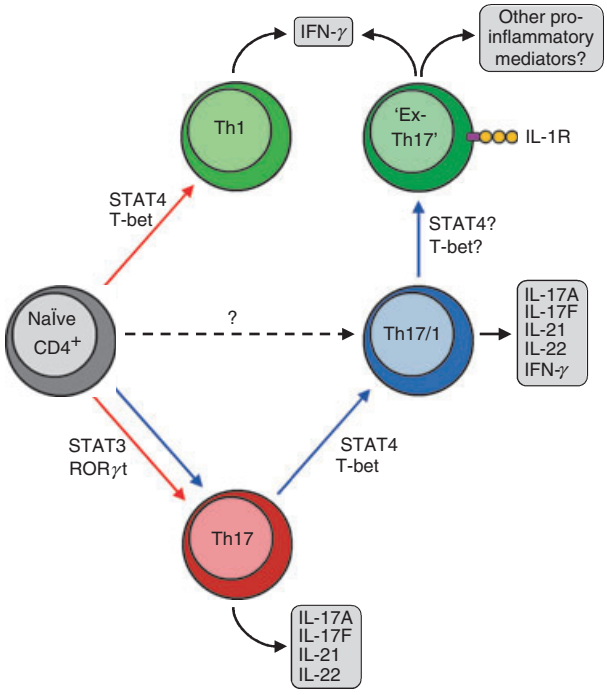

Figure 3. Do signal transducer and activator of transcription 3 (STAT3), STAT4, nuclear retinoic acid-related orphan receptor (ROR)γt, and T-bet have to be expressed in the same CD4$^+$ T cell to induce a colitogenic phenotype? CD4$^+$ T cells deficient in STAT3, STAT4, RORγt, or T-bet fail to induce colitis after transfer to T-cell-deficient mice, indicating that factors important for either T helper 1 (Th1) or Th17 differentiation are required to induce intestinal inflammation. However, it is not known exactly where at the stage of differentiation these STAT proteins and transcription factors are required for disease induction. We suggest two alternative hypotheses to explain why a deficiency in only one of these four key regulators renders T cells unable to induce colitis. In the first scenario (red arrows), STAT4/T-bet are required for Th1 development and STAT3/RORγt for Th17 cell differentiation, and a mixture of these two populations are needed to trigger inflammation. In the second scenario (blue arrows), STAT3/RORγt are required for the initial Th17 cell differentiation, and STAT4/T-bet for the subsequent phenotype switch of that cell towards an interferon-γ (IFN-γ)$^+$ interleukin-17A (IL-17A)$^+$ and later an IFN-γ^+ 'ex-Th17' cell. These 'ex-Th17' cells show similarities to Th1 cells, but they also display distinct characteristics such as high levels of aryl hydrocarbon receptor (AhR) mRNA and surface IL-1 receptor (IL-1R) expression. 'Ex-Th17' cells may also secrete other pro-inflammatory mediators not produced by Th1 cells, thereby contributing to pathology. Determining exactly when the key STAT proteins and transcription factors are acting at the single-cell level during effector T-cell development in colitis may help shed light on the specific contribution of Th1, Th17, Th17/1, and 'ex-Th17' cells to intestinal pathology.

expression and turned on IFN-γ are identical to true Th1 cells at the cellular and molecular level. Recent evidence suggests that differences do exist, as in contrast to Th1 cells, IFN-γ^+ 'ex-Th17' cells maintain high levels of aryl hydrocarbon receptor (AhR) mRNA and cell surface IL-1R.[91] By using reporter mice and cell separation based on cytokine secretion profile and IL-1R1 expression it should in the future be possible to further characterize these two populations, as well as the IFN-γ/IL-17A double producers, to examine these cells in more detail and look for other factors that may be involved in disease pathogenesis.

Taken together, Th17-type responses can have both pathogenic and disease-protective roles in the intestine depending on the setting and microenvironment. Many questions still remain to be answered before we will fully understand colitis pathogenesis, one of them being that of Th17 plasticity and the role of these cells and their cytokines in the intestine. Hopefully new knowledge gained from experimental models will help to shed light on these and other related questions, resulting in information that subsequently will be translated to and help our understanding of the even more complex scenario of human IBD.

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Note added in proofs

Since our review was accepted for publication, an article has been published describing IL-23-responsive innate lymphoid cells in the human intestine, a cell population that expresses Th17-associated cytokines and that is increased in the inflamed intestine of Crohn's disease patients (Geremia *et al., J Exp Med* 2011; May 16, Epub ahead of print).

Disclosures

The authors have no conflicts of interests.

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